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Low Serum Vitamin D and Normal Serum 8-Hydroxy-2-Deoxyguanosine Levels in Endometrioma Patients: Is It a Dilemma?

Endometrioma Hastalarında Düşük Serum Vitamin D ve Normal Serum 8-Hidroksi-2-Deoksiguanosin Seviyeleri: Bu Durum İkilem mi?

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ÖZ

Amaç: Endometrioma hastalarında serum 25-OH vitamin D ve 8-Hidroksi 2-deoksigunazin (8-OH2dG) seviyelerini karşılaştırmak, kist boyutu ile ilişkisini saptamak.

Gereç ve Yöntemler: Çalışmaya endometrioma tanısı alan 54 hasta ile normal over dokusuna sahip 26 hasta kontrol grubu olarak dahil edildi. Serum örneklerinde 25-OH vitamin D ve 8-OH2dG seviyelerine bakıldı.

Bulgular: Hasta ve kontrol grubu arasında yaş, gebelik, doğum sayısı ve vücut kitle indexi (VKI) bakımından farklılık yoktu. Hasta grubunun D vitamini düzeyi, kontrol grubuna göre anlamlı derecede düşüktü (p=0.001). Kisti bilateral olan hastalar ile, kisti unilateral olan hastalar arasında D vitamini düzeyleri bakımından anlamlı farklılık yoktu (p=0.39). Hasta grubunda 48 (%88.8), kontrol grubunda 11 (%42.3) hastada D vitamini seviyesi <30-microgr/L idi. Hasta grubunda, D vitamini düzeyi hipovitaminoz seviyede olan hastalarda ortalama endometrioma çapı 58,4±25,14 mm iken, D vitamini düzeyi normal saptanan hastalarda endometrioma çapı ise 35,33±18,26 mm idi. Hipovitaminoz D saptanan hastalarda kist çapı ile D vitamini düzeyleri arasında ters bir ilişki saptandı. İki grup arasında 8-OH2dG düzeyleri bakımından anlamlı farklılık yoktu (p>0.05).

Sonuç: Endometrioma varlığı, kist çapı ve serum D vitamini düzeyleri arasında ters ilişki olmasına rağmen, serum 8-OH2dG düzeylerinde değişiklik yoktu. Bu durum, overyan endometriozisin patogenezinde hala belirsiz noktalar olduğunu göstermektedir.

Anahtar Kelimeler: Endometrioma, 25-OH vitamin D, 8-hidroksi 2-deoksiguanozin

ABSTRACT

Aim: To compare serum 25-OH vitamin D and 8-Hydroxy 2-deoxyguanosine (8-OH2dG) levels in endometrioma patients and to determine their relationship with cyst sizes.

Material and Methods: 54 patients with an endometrioma, 26 patients with normal ovarian tissue were included in the study. 25-OH vitamin D and 8-OH2dG levels were measured in serum samples.

Results: There were no differences between the patient and control groups in terms of age, pregnancy, number of births and body mass index (BMI). Vitamin D levels of the patient group were significantly lower than the control group (p = 0.001). There was no significant difference in terms of vitamin D levels between patients with bilateral and unilateral cysts (p = 0.39). Fourty-eight (88.8%) patients in the patient group and 11 (42.3%) patients in the control group had a vitamin D levels of <30 microgr/L. Cyst diameter was 35.33 ± 18.26 mm in patients with normal vitamin D levels, while cyst diameter was 58.4 ± 25.14 mm in patients with hypovitaminosis D in the patient group. An inverse relationship was found between the cyst diameter and vitamin D levels in patients with hypovitaminosis D. There was no significant difference between the two groups in terms of 8-OH2dG levels (p> 0.05).

Conclusion: Although there was an inverse relationship between the presence of endometrioma, cyst diameter and serum vitamin D levels, there was no change in serum 8-OH2dG levels. This suggests that there are still unclear spots in the pathogenesis of ovarian endometriosis.

Keywords: Endometrioma, 25-OH vitamin D, 8-hydroxy 2-deoxyguanosine

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INTRODUCTION

Endometriosis is an estrogen-dependent inflammatory disease characterized by the presence of endometrial tissue outside the uterine cavity, associated with pelvic pain and infertility (1,2). It affects approximately 10% of all women and 40% of infertile women (3). Endometriosis occurs through a complex interaction of immunological, hormonal, genetic and environmental factors; however, the etiology is not entirely clear (2).

Immunomodulatory, anti-inflammatory and antiproliferative properties of vitamin D suggest a possible role in the pathogenesis of endometriosis (4). Recent studies have shown that vitamin D receptor (VDR) and 1α-hydroxylase expression are present in the endometrium and thus vitamin D plays a role in endometriosis pathogenesis (5).

It has been shown that oxidative stress, defined as the imbalance between reactive oxygen species (ROS) and antioxidants, plays a role in the pathophysiology of endometriosis leading to an inflammatory response in the peritoneal cavity (6-8). Ovarian endometrioma, one of the most common forms of endometriosis, is characterized by the presence of one or more cysts covered with endometrial tissue. A good measure of evidence supports the view that endometriotic cysts can have deleterious effects on the surrounding ovarian microenvironment, thus creating a risk for the functionality of adjacent follicles (9).

Most of the studies in the literature are related to the role of vitamin D and oxidative stress in endometriosis. The number of studies on the role of vitamin D and oxidative stress on endometrioma cysts is limited. This study intends to evaluate the levels of vitamin D and 8-hydroxy-2-deoxyguanosine (8-OH-2dG) which is a sensitive indicator of DNA damage in the serum of patients with endometrioma cysts, and demonstrate whether or not they correlate with cyst sizes.

MATERIAL AND METHODS

Fifty-four patients of reproductive age who were admitted to our clinic between March 2017- September 2018 and diagnosed with endometrioma during a routine ultrasonographic examination, and who were with normal weight (18.5-24.9 kg / m2) according to their body mass index (BMI), were included in the control group as well as 26 patients with a normal ovarian structure according to an ultrasonographic evaluation compatible with the age and BMI patient group. The study was approved by the Clinical Trials Ethics Committee of Gaziantep University (2017/45). A signed informed consent form was obtained from each patient who agreed to participate in the study.

Patients who had an endometrioma or ovarian cyst surgery, were previously diagnosed with extra ovarian endometriosis (adenomyosis, pelvic endometriosis) or who had other gynecological diseases (polycystic ovary syndrome, myoma), as well as those receiving medical treatment (D vit. replacement, oral contraceptive), or who have had intense exposure to the sun within the last 3 months and have a systemic disease and smoke, were not included in the study. The ages of the patients, the number of pregnancies and births, BMIs, unilaterality-bilaterality status and the size of cysts were recorded.

Ultrasonographic evaluation of the patients diagnosed with an endometrioma was confirmed transvaginally by the same gynecologist using the Voluson E8 ® (GE Healthcare) device with a 3.5-5.5 MHz probe. Endometrioma was defined as an ovarian cyst in the icy glass echogenicity, diffuse-homogeneous and without solid area (10). The anteroposterior and right-left diameters of the cyst were measured three times and the mean measurement value was recorded.

Blood samples were taken in August 2017 to minimize seasonal changes in vitamin D levels between the patient and control groups considering our country has ample sun. After taking 4-5 cc venous blood, samples were centrifuged at 4000 rpm for 10 minutes. The serum was then removed and placed in labeled eppendorfs and stored at -80 ° C until later use.

The Northwest Kit (Northwest, NWLSS 8-OHdG ELISA High Sensitivity Kit, Vancouver, Canada) was used to measure the amount of 8-OH2dG in the serum, which is a marker of oxidative DNA damage.

Serum samples stored at -80 °C were brought to room temperature before starting the study. Each dissolved serum was passed through Millipore Microcon Centrifugal Filters (cutoff 10,000) to remove large molecular weight substances. Each serum was placed in 2 mL filters and centrifuged at 14000 rpm for 10 minutes. The results were expressed in pg/mL.

25-OH vitamin D measurement was performed with a Beckman Coulter Uni-Cel DxI 800 Access Immunoassay System device using a two-stage competitive immunoenzymatic method. The results were expressed in microgr/L. Serum vitamin D levels of ≥ 30 microgr/L were accepted as normal, while <30 microgr/L levels were regarded a representing a case of hypovitaminosis.

STATISTICAL METHOD

Normal distribution of numerical variables was tested using the Shapiro-Wilk test. The Student's t-test was used in the comparison of normally distributed variables between both groups, and the Mann-Whitney U test was used to compare variables that were not distributed normally between both groups. ANOVA and LSD multiple comparison tests were used to compare variables with normal distribution between three groups, whereas the Kruskal-Wallis and All-pairwise multi-comparison tests were used for the comparison of variables with non-normal distribution between three groups. SPSS 22.0 version for Windows was used in the analyses, and $p<0.05$ was considered significant.

RESULTS

There were no differences between the patient and control groups in terms of age, pregnancy, number of births and BMI (Table 1).

Table 1: Comparison of numerical measurements in patient/control groups

Variables	Patient (n=54)	Control (n=26)	P
Age	28.57 ± 6.56	26.23 ± 7.31	0.057
Gravida	0.98 ± 1.46	1.38 ± 1.5	0.248
Parity	0.65 ± 1.15	0.92 ± 1.09	0.142
BMI (kg 1m²)	21.98±1.81	21.19±1.65	0.060

BMI: body mass index

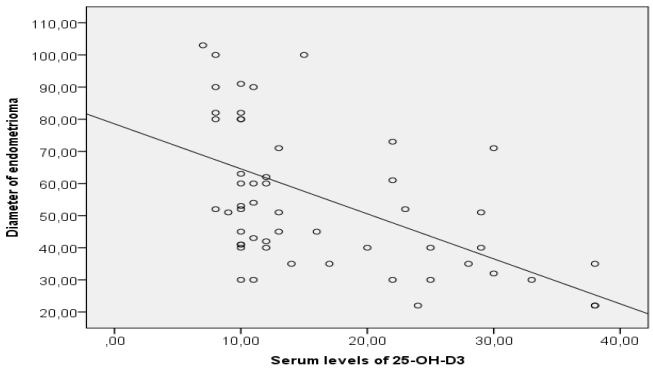
The mean cyst diameter in the patient group was 55.83 mm (min: 22 mm, max: 150 mm). The cyst was in the right ovary in 23 (42.6%) of the patients, the left ovary in 21 (38.9%) of the patients and bilateral in 10 (18.5%) of the patients.

The mean level of vitamin D was 20.82 microgr/L (min: 7.44, max: 63.52). Fifty-nine (73.8%) patients had vitamin D hypovitaminosis (<30 microgr/L). Fourty-eight (81.35%) of the patients with hypovitaminosis were in the patient group and 11 (18.64%) were in the control group. Vitamin D level was 16.25 ± 8.9 microgr/L in the patient group and 30.33 ± 11.72 microgr/L in the control group. Vitamin D levels of the patient group were significantly lower than those of the control group ($p = 0.001$). There was no significant difference in

terms of vitamin D levels between the 10 patients with bilateral cysts and the 44 patients with unilateral cysts (p = 0.39).

Fourty-eight (88.8%) patients in the patient group and 11 (42.3%) patients in the control group had a vitamin D levels of <30 microgr/L. Cyst diameter was 35.33 ± 18.26 mm in patients with normal vitamin D levels, while cyst diameter was 58.4 ± 25.14 mm in patients with hypovitaminosis D in the patient group. There was a statistically significant difference between the patients with low and normal vitamin D levels in terms of cyst diameter (p = 0.007). A linear correlation was observed between a decrease in vitamin D levels and the cyst diameter (r = -0.584, p = 0.001) (Figure 1).

Figure 1: The relationship between vitamin D levels and cyst diameter



Demographic data of patient groups with and without hypovitaminosis D are summarized in Table 2.

Table 2: Demographic data of patients with low or normal levels of vitamin D in patient group

	D vit level (n=54)		
	<30 (n=48)	**≥ 30 (n=6)**	**p**
Age	27.83±6.59	27.76±7.72	0.822
BMI	21.82±1.82	21.44±1.70	0.386
Gravida	1.10±1.45	1.14±1.59	0.765
Parity	0.71±1.11	0.81±1.21	0.980

The mean 8-OH2dG level in the patient group was 454.44 ± 127.7 pg/mL and 469.57 ± 120.07 pg/mL in the control group. There was no significant difference between the two groups in terms of 8-OH2dG levels (p> 0.05).

DISCUSSION

Endometriosis is thought to be a multifactorial disease (11,12). In particular, local inflammation processes seem to play a role in the development and progression of the disease (13).

The biological mechanisms through which vitamin D levels may influence the risk of endometriosis is not yet fully understood, but vitamin D may play a role in disease pathogenesis via the regulation of immunological functions (14). In women with endometriosis, a change in immune surveillance has been defined in the form of depletion and increased humoral immune response in cell-mediated immunity (15). Therefore, vitamin D may affect the development and progression of endometriosis with its role in reducing proinflammatory processes.

Only a few in vivo studies have investigated the potential correlation between vitamin D serum levels and endometriosis, showing contrasting results (16-18). In a study conducted by Buggio et al., no statistically significant difference was observed in the 25 (OH) vitamin D serum levels of women with endometriosis and without endometriosis. In addition, there was still no difference after classifying patients into phenotypic categories of deep endometriotic lesions and ovarian endometriomas (19). In contrast to this study, some authors have suggested that there is a correlation between endometriosis and hypovitaminosis D (16,17), and more recently, higher vitamin D serum levels are associated with a lower risk of endometriosis (20).

In a cohort study by Ciavattini et al., relatively high levels of hypovitaminosis D were found in women of reproductive age diagnosed with ovarian endometriosis (10). Age, BMI, genetic factors and lifestyle have been shown to be associated with increased risk of vitamin D deficiency (21,22). However, no significant difference was found between women with normal vitamin D serum levels and women with hypovitaminosis D in terms of age, BMI and clinical features. Interestingly, although the mean diameter of the ovarian endometrioma was similar in both subgroups (40.2 ± 22.6mm in women with hypovitaminosis D vs 26.7 ± 12.1mm in women with normal vitamin D serum level; p1 / 40.1), a significant linear correlation emerged between 25-OH vitamin D serum levels and ovarian endometrioma diameter, and this correlation remained with correction of variables in multivariate logistic regression (age, BMI, smoking, menarche age and number of pregnancies) (20).

In this study, we established no difference between the patient and control groups in terms of age, BMI, pregnancy and number of births. However, there were significant differences in vitamin D levels between the two groups. Vitamin D levels in the patient group were lower than those in the control group (16.25 ± 8.9 vs 30.33 ± 11.72 microgr/L, p = 0.001). After classifying the patient group into two groups, those with hypovitaminosis (n = 48/54) and those without hypovitaminosis (n = 6/54), the cyst diameter was higher in the group with low vitamin D levels (58.4 ± 25.14 mm vs 35.33 ± 18, 26 mm, p = 0.007). A linear correlation was observed between a decrease in vitamin D levels and an increase in the cyst diameter (r = -0.584, p = 0.001). In the study conducted by Ciavattini et al., (20) vitamin D levels were evaluated only in the patient group without any control group and a relationship between vitamin D and cyst diameter was found in patients with hypovitaminosis. Whereas in our study, after comparing the vitamin D levels between the patient and control groups, we examined the relationship between cyst diameter and vitamin D levels among groups in the patient group with and without hypovitaminosis. Thus, we found that hypovitaminosis D was more prevalent in patients with endometrioma and there was a linear relationship between the degree of hypovitaminosis D and the cyst diameter.

A recent in vitro study found that treatment of endometrial stromal cells with 1.25 (OH)2 D3 significantly reduced IL-1b or TNF-a-induced inflammatory responses such as mRNA expression, prostaglandin activity and matrix metalloproteinase expression (16). In 2013, Abbas et al. demonstrated that vitamin D treatment provided a reduction in endometrioma cyst size in a rat surgical model, resulting in apoptosis as well as fibrosis in the stroma, suggesting that vitamin D administration may have a beneficial effect in the treatment of endometriosis (23).

Anastasi E. et al. showed that women with endometriosis had lower vitamin 25-OH vitamin D levels than healthy women of reproductive age. In addition, insufficient levels of vitamin D were significantly correlated with the presence of moderate/severe pelvic pain in this study. This finding may be related to the lack of local inflammation modulation due to low vitamin D levels (24). 1,25 (OH) vitamin D exhibits an important antineoplastic activity that affects cell growth, differentiation, and apoptosis, possibly affecting endometriosis growth (25).

As shown in the above studies, the potential correlation between vitamin D serum levels and endometri oma, which our study also supports, may lead to the development of new therapeutic strategies for the disease. Vitamin D supplementation may be a new, safe and cost-effective treatment strategy for both the prevention and treatment of endometrioma in women with hypovitaminosis D. However, well-designed, large, randomized controlled trials are needed to properly assess the possible therapeutic role of vitamin D in women with endometrioma and identify the safe dosage.

In recent years, the literature has shown that oxidative stress, as well as vitamin D, play a role in the pathogenesis and progression of endometriosis (26).

Free radicals play a role in the pathogenesis of many diseases, but data on the role of oxidative stress in the development of endometriosis are still controversial. The imbalance between radical-forming and radical-solvent systems in the peritoneal cavity of patients with endometriosis can potentially be induced by increased erythrocytes, apoptotic endometrial cells, and peritoneal fluid macrophages (27). In the literature, levels of oxidative stress markers were checked in serums, follicular fluid (FF), peritoneal fluid (PF) and tissue. 8-OH2dG levels, which are one of the oxidative stress markers, were mostly examined in the tissue and were found to be higher in samples with endometriosis (28).

The 8-OH2dG concentrations, a sensitive indicator of DNA damage caused by free radicals, was found to be higher in the normal ovarian cortex surrounding the endometriomas compared to the tissue surrounding the serous and dermoid ovarian cysts in a study by Matsuzaki et al (29). This finding shows that endometriotic cysts cause oxidative stress in the adjacent healthy tissue. In a study conducted by Kao et al., 8-OH2dG and lipid peroxide levels were found to be 6 times higher in the endometriotic cyst than non-endometriotic ovarian cysts (30).

While in a study conducted by Mizhele et al. in 2016, 8-OHdG levels from the serum samples of patients with endometriosis did not differ from those of the control group, there were higher concentrations of 8-OH2dG in the FFs. This is suggestive of OS formation in the microenvironment of infertile patients with the disease who are administered controlled ovarian stimulation (COS) (31). Seino et al. (32) have shown that infertile women with high 8-OH2dG concentrations in their granulosa cells who have received in vitro fertilization (IVF) have a lower fertilization rate and embryo quality. Supporting these conclusions, Tamura et al. (33) have shown that a higher 8-OH2dG concentration in the FF of women being administered IVF correlate with a higher degree of degenerated oocytes. This suggests that 8-OH2dG in the follicular compartment has a toxic effect on the oocyte.

In this study, we have examined 8-OH2dG levels in serum samples belonging to patients with endometrioma and the control group with normal ovarian tissues. However, we have not found a significant difference between the patient and control groups in terms of 8-OH2dG levels (454.44 ± 127.7 pg/ mL vs 469.57 ± 120.07 pg/ mL). When we look at the literature, the number of studies checking the 8-OH2dG levels in the serum of endometrioma patients is limited. As mentioned above, in most of the studies in the literature the levels of this marker were evaluated in patients with endometriosis and 8-OH2dG levels in the serum samples were not different between the patient and the control groups, as in our study (31). Interestingly, while there were no differences in the 8-OH2dG levels in the serum samples of the same patients, 8-OHdG levels were found to be higher in the FFs. 8-OH2dG levels of the endometrioma cyst or its contents were found to be higher in the patient groups (29,30).

In these cases, antioxidant supplementation was suggested as an urgent intervention to prevent damage to the ovarian tissue and maintain oocyte efficiency (34). However, it is necessary to clarify why 8-OH2dG levels differ in the serums and peritoneal fluids of the same patients in order to make such an interpretation.

Small number of patients may be the limitation of our study. In order to determine the relationship between vitamin D and 8-Oh2dG levels and endometrioma, there is a need for studies on more patients in both serum and tissue samples.

Based on the knowledge that 8-OH2dG is an oxidative stress marker and increased inflammatory response occurs in an environment with increased oxidative stress, we thought it best to correlate 8-OH2dG levels and anti-inflammatory 25 (OH) vitamin D levels in endometrioma patients while designing the study. However, in our study, we did not find any differences between patient and control groups in terms of 8-OH2dG levels although we found that endometrioma patients had significantly lower levels of 25 (OH) vitamin D compared to the control group and that this correlated with the cyst diameter. Although this result may be due to our patient group being limited, it may also highlight there are still be unexplored spots in the pathogenesis of the disease. The fact that 8-OH2dG levels are similar in serum samples and higher in tissue samples of endometrioma / endometriosis patients in the literature may suggest that it is only a local oxidative stress response.

We think that larger, randomized and multicenter studies are needed to reveal the relationship between vitamin D and 8-OH2dG in patients with endometrioma, and to offer an opinion on whether vitamin and/or antioxidant therapy can be used in the prevention and treatment of the disease.

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